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Comparison of neutrophils lymphocyte ratio in type-2 diabetes mellitus patients and healthy individuals

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Abstract--Diabetes mellitus is the disease of concern in almost all part of the world including India and its clinical condition characterized by chronic hyperglycaemia. Neutrophils lymphocyte ratio is a relatively newer parameter that has been recently considered as alternate to assess the underlying inflammation and it can be easily determined with the help of complete blood count report. The aim of this study was to observe the status of NLR ratio in newly diagnosed type -2 diabetes mellitus patients and healthy individuals in Indian population. A comparative cross-sectional study was conducted in subjects between 19-60 years of age. A total of 216 age matched type-2 diabetes mellitus patients and 216 healthy individuals were selected for the study. All subjects were investigated for complete blood count, (CBC), Fasting and Postprandial blood sugar, and Glycated Hemoglobin (HbA1c) in hospital lab services. This study has been conducted in the Department of Physiology in collaboration with Department of Medicine, and Hospital Lab Services after having

clearance from the institutional ethical committee at Maharaja Suheldev Autonomous State Medical College and Maharshi Balark Hospital bahraich, uttar Pradesh. A positive correlation between Neutrophils lymphocyte ratio and type-2 DM, so NLR should be used as prognostic and disease monitoring tool during the follow up diabetic patients like HbA1C.

Keywords---NLR, IDF, CBC, ROC, and HbA1C.

Introduction

Diabetes mellitus is the disease of concern in almost all part of the world including India. Diabetes mellitus is a clinical condition characterized by chronic hyperglycaemia. In hyperglycaemia, there is increased nonenzymatic glycation of proteins and the rate of the glycation proportionately depends upon the blood level of glucose. Thus, there is increased formation of glycated proteins due to hyperglycaemia [1]. HbA1c and glycated albumin are the examples of glycated protein which are used in diagnosis of diabetes and assessing the status of hyperglycaemia. These glycated proteins cause activation of pro-inflammatory mediators. These mediators produce chronic inflammation in the body [2]. This chronic inflammation causes increased activity of neutrophils which is key inflammatory cell involved in atherosclerosis which is one of the most important pathological process that leads to endothelial dysfunction and later is said to be responsible for chronic complications and cardiovascular events like stroke and Myocardial infarction in diabetes [3].

This increased chronic inflammation causes increased production of inflammatory markers like CRP and depletion or exhaustion of antioxidant system of the body, a condition known as oxidative stress. This increased oxidative stress is said to be main culprit responsible for cardiovascular events and other complications occurring in the late half of the disease [4,5] Various tests are available to estimate the oxidative stress in the body like hs CRP, IL-6, TNF- α , and many others. But these tests are expensive and are not easily available at all centers.

Neutrophils lymphocyte ratio is a relatively newer parameter that has been recently considered as alternate to assess the underlying inflammation. NLR ratio can be easily determined with the help of complete blood count report. CBC is a basic investigation being done in almost all patients with suspected infection and inflammation. CBC test is easily available and it is not a costly test. So, NLR ratio can be easily calculated from CBC without any extra burden on the patients. Many studies have been done showing increased neutrophils lymphocyte ratio is a marker of disease related inflammation [6]. Few studies also reported that NLR ratio is linked with higher HbA1c and poor glycemic control in diabetes mellitus type-2 [7]. Zahorec R et al [8] stated NLR meter in their review article. NLR ratio range 1-2 is said to be normal range, 2-3 indicate low inflammation and stress, 3-7 indicates mild to moderate inflammation, 7-11 indicated moderate inflammation with stress and >11 indicates severe inflammation and stress.

Several studies have observed that uncontrolled diabetes is associated with high NLR ratio. Mazhar Hussain et al ^[9] observed that subjects with normal TLC but increased NLR ratio had increased risk of atherosclerosis which is a key pathological process of cardiovascular complications in diabetics. Heng Wan et al ^[10] observed increased prevalence of CVD and diabetic kidney disease in subjects with high NLR ratio. SM Ulu et al ^[11] observed higher NLR ratio as a remarkable marker in diabetic retinopathy evaluation. Seema Rahar et al ^[12] found NLR ratio as a novel and inexpensive marker of systemic inflammation in diabetes that also correlates well with severity of diabetic nephropathy.

We have seen that many studies has been done to compare NLR with glycemic control and chronic complications of diabetes but very limited data is available on NLR ratio in new onset diabetes mellitus. Therefore, we planned this study to observe the status of NLR ratio in newly diagnosed diabetes mellitus patients in Indian population.

Methodology

This study has been conducted in the Department of Physiology in collaboration with Department of Medicine and Hospital Lab Services after having clearance from the institutional ethical committee. Total 432 (216 cases and 216 controls) subjects of both the genders between 19-60 years of age had been recruited from the medicine Out-Patient of Department of Maharaja Suhel dev Autonomous State Medical College and Maharshi Balark Hospital. Subjects with known chronic respiratory disease, cardiovascular diseases, endocrinal disease, hepatic or renal disease and psychiatric illness were excluded from the study. Similarly, pregnant females, lactating women, females with gynaecological problems were also excluded from the study.

All recruited subjects were asked for clinical history, family history in detail. All subjects were investigated for complete blood count, (CBC), Fasting and Postprandial blood sugar, Glycated Haemoglobin (HbA1c), and C-Reactive protein. Subjects having diabetes were put into case group (n=216) and subjects with normal blood sugar were put into control group. Then, various blood cells and NLR ratio was compared and statistically analysed in both the groups.

RESULTS:

Table-1: Distribution of gender between the groups

Gender	Cases (n=216)		Controls (n=216)		p-value ¹
	No.	%	No.	%	
Male	141	65.3	139	64.4	0.84
Female	75	34.7	77	35.6	

¹Chi-square test

Table-1 shows the distribution of gender between the groups. More than half of both cases (65.3%) and controls (64.4%) were males. There was no significant ($p>0.05$) difference in gender between the groups showing comparability of the groups in terms of gender.

Table-2: Comparison of anthropometric parameters between the groups

Anthropometric parameters	Cases (n=216)	Controls (n=216)	p-value ¹
Height in cms	163.01±8.34	164.03±8.25	0.20
Weight in kgs	62.56±8.47	63.39±9.43	0.33
BMI in kgs/mtr ²	23.49±2.73	23.53±2.63	0.86

¹Unpaired t-test

Table-2 shows the comparison of anthropometric parameters between the groups. There was no significant ($p>0.05$) difference in anthropometric parameters between the groups.

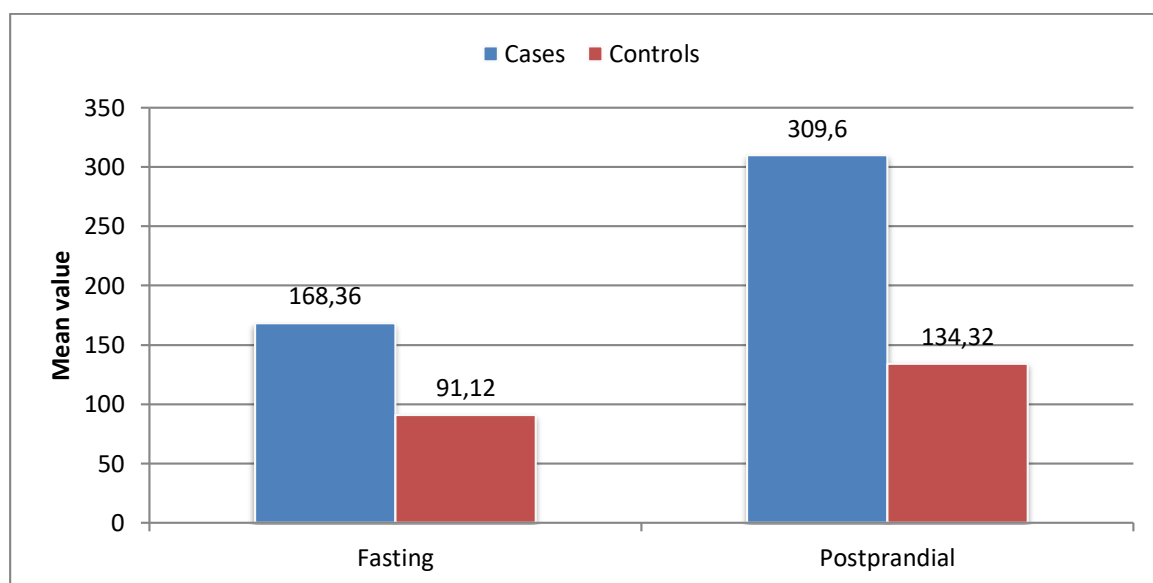


Figure- 1 Comparison of Blood sugar between the groups

Fig.1 shows the comparison of blood sugar between the groups. Both fasting and postprandial were significantly ($p=0.0001$) higher among cases compared to controls.

Table-3: Comparison of HbA1c between the groups

Groups	HbA1c (Mean±SD)
Cases	8.17±0.89
Controls	5.30±0.56
p-value ¹	0.0001*

Table-3 shows the comparison of HbA1c between the groups. HbA1c was significantly ($p=0.0001$) higher among cases (8.17 ± 0.89) compared to controls (5.30 ± 0.56).

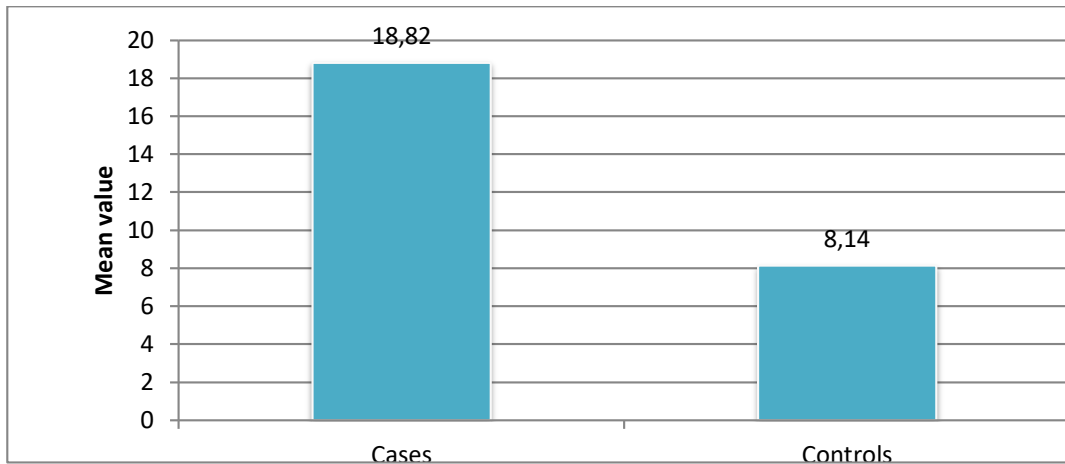


Figure: 2 - Comparison of CRP between the groups

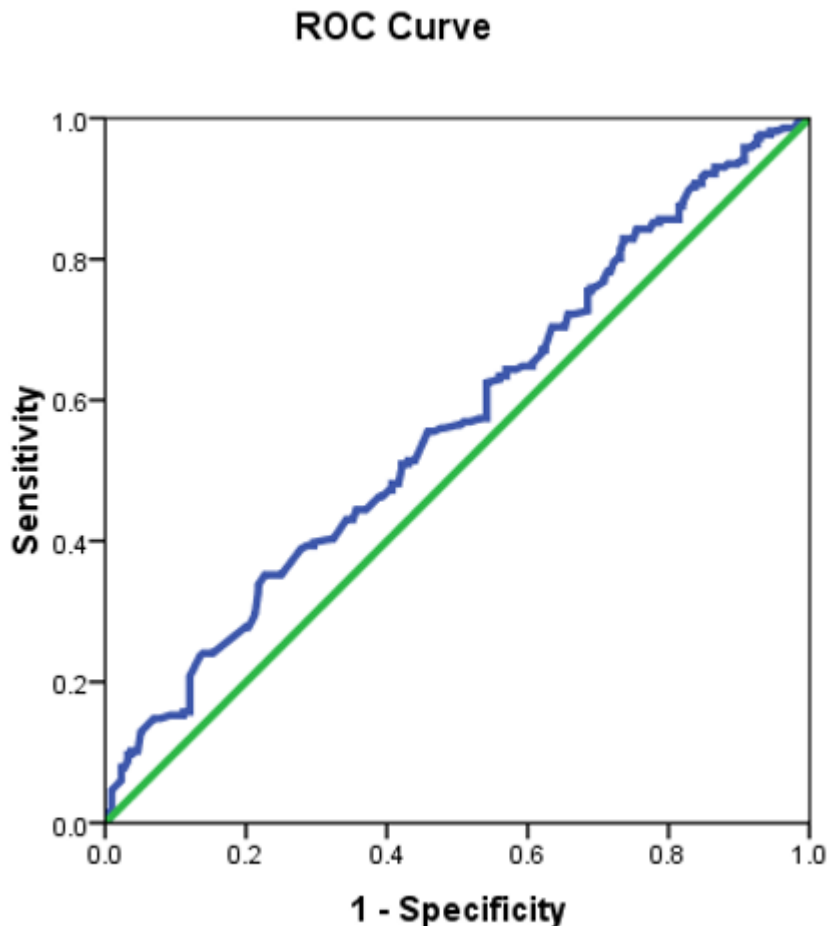
Figure.2- shows the comparison of CRP between the groups. CRP was significantly ($p=0.0001$) higher among cases (18.82 ± 4.76) compared to controls (8.14 ± 4.21).

Table-4: Comparison of Neutrophils, lymphocyte & NLR between the groups

DLC	Cases (n=216)	Controls (n=216)	p-value ¹
Neutrophils	66.23 ± 3.67	63.38 ± 6.34	0.001*
Lymphocyte	26.66 ± 3.62	28.26 ± 4.60	0.001*
Neutrophils- Lymphocyte ratio	2.54 ± 0.42	2.42 ± 0.44	0.001*

¹Unpaired t-test, *Significant

Table-4: shows the comparison of neutrophils, lymphocyte between the groups. Neutrophils was significantly ($p=0.001$) higher among cases (66.23 ± 3.67) compared to controls (63.38 ± 6.34). Neutrophils- Lymphocyte ratio was also significantly ($p=0.001$) higher among cases (2.54 ± 0.42) compared to controls (2.42 ± 0.44). However, Lymphocyte was found to be significantly ($p=0.001$) lower among cases (26.66 ± 3.62) compared to controls (28.26 ± 4.60).



Diagonal segments are produced by ties.

Fig. 3: ROC curve showing sensitivity and specificity of Neutrophils -Lymphocyte ratio in predicting diabetes mellitus

Figure.3: ROC curve analysis was used to get the cut-off point of NLR. The cut-off point of NLR was 2.5 and the area under the curve (AUC) was 0.56 (95% CI, P-value 0.01)

Table-5: Predictive value of Neutrophils -Lymphocyte ratio in predicting diabetes

Neutrophils - Lymphocyte ratio cutoff	Cases		Controls		Total	
	No.	%	No.	%	No.	%
>2.5	121	28.1	104	23.9	225	52.0
≤2.5	95	22.0	112	26.0	207	48.0
Total	216	50.1	216	49.9	432	100.0
Predictive values, %						

AUC, p-value	0.56, 0.01*
Sensitivity	56.0
Specificity	51.9
PPV	53.8
NPV	54.1

%ages from total no. of cases and controls, *Significant

Table.5: shows that 121 subjects with diabetes mellitus were having NLR ratio >2.5 and 95 diabetics had their NLR ration <2.5. It also shows that 104 non diabetics had NLR ratio >2.5 and 112 non-diabetics had their NLR ration <2.5.in the present study, the sensitivity, specificity, positive predictive value of NLR ratio to diagnose diabetes mellitus is 56%, 51.9%, 53.8 % and 54.1 % respectively.

Discussion

We have selected subjects from the medicine OPD on the basis of inclusion and exclusion criteria. All subjects were screened for diabetes as per WHO criteria. All those having diabetes were kept in case group and rest were put in control group. Thus, FBS, PP blood sugar and HbA1c values were higher in case group. In euglycemic conditions, various proteins present in blood undergo non-enzymatic glycosylation. Glycosylated haemoglobin (HbA1c) is a typical example of this non enzymatic glycosylation. We know that diabetes is a condition characterized by hyperglycaemia. This prolonged or chronic hyperglycaemia causes increased non enzymatic glycosylation of proteins leading to formation of advanced glycated products (AGPs). These AGP causes activation of neutrophils. Neutrophils are first line defence against pathogens and during inflammation which releases various proinflammatory factors [2]. This results in further increase in inflammation (chronic inflammation) and neutrophilia. In the present study, we observed an increase in neutrophils count in persons with type-II diabetes mellitus (66.23 ± 3.67) in comparison to non-diabetics (63.38 ± 6.34). This increase in neutrophils in diabetics was statistically significant.

Diabetes is clinical condition associated with chronic stress [13]. Chronic stress is associated with lymphopenia. Margination and redistribution of lymphocytes are mainly responsible for lymphopenia in peripheral venous blood. In our study, we have observed lower lymphocytes (26.66 ± 3.62) in case group in comparison to control group (28.26 ± 4.60) and this difference was statistically significant.

Increase in neutrophils count and decreased lymphocyte count leads to increased neutrophil-lymphocyte ratio in subjects with diabetes mellitus. Neutrophils are part of innate immunity and lymphocytes are responsible for acquired immunity. Therefore, NLR ratio indicates balance between innate immunity and adaptive immunity. A rise in NLR ratio indicates shift of adaptive immunity to innate immunity [8]. We already know that inflammation causes neutrophilia and stress causes increase in serum cortisol levels which leads to lymphopenia. Thus, increased NLR ratio is indicative of inflammation and stress together. In diabetes mellitus, there is hyperglycemia that causes increased formation of advanced glycation products which in turn results in increased inflammation and neutrophilia. Diabetes is associated with chronic stress that may increase the

cortisol levels and this causes lymphopenia. This might be the explanation of increased NLR ratio in diabetes subjects.

In our study, we noticed higher NLR (2.54 ± 0.42) ratio in diabetics than non-diabetic control group (2.42 ± 0.44) and the difference was statistically significant. Lou, M et al ^[14] reported significantly higher NLR ratio was associated with insulin resistance in newly diagnosed type 2 diabetics. They also proposed raised NLR as a predictive marker of insulin resistance. Sefil ^[15] reported raised NLR may be associated with high HbA1c type 2 diabetics. Akin, S et al ^[16] suggested that NLR ratio might be used as an easily measurable, noninvasive, and cost-effective parameter for the follow-up of diabetic patients.

As per NLR meter given by Zahorec et al ^[8], NLR ratio between 2-3 indicate low inflammation and stress. In our study, NLR ratio in control group is 2.42 ± 0.44 indicating low grade inflammation with stress even in control group. Deepak Y. Kamath ^[17] et al found in their study that hsCRP level in control group had higher mean hsCRP value in comparison to western population indicating low level of systemic inflammation in healthy adults. Therefore, a larger multicentric study should be done to detect normal range of NLR ratio in Indian population. It is also observed that 121 diabetics has their NLR ratio >2.5 . Thus, 56% of diabetics were having NLR ratio >2.5 . Similarly, we also observed that 112 non diabetics were having their NLR ratio <2.5 . In this study, the sensitivity and specificity of NLR ratio to detect type 2 diabetes comes out to be 56% and 51.9% respectively.

We can conclude from this study that high NLR ratio is associated with the type-II diabetes mellitus. Diabetes is emerging as a major health issue in India and the onset of the disease is approx. one decade earlier in comparison to western world. Therefore, high suspicion is required to diagnose diabetes in maximally. As complete blood count (TLC & DLC) is a very common cheap and easily available test, NLR ratio can be easily estimated and NLR ratio >2.5 can be used as an indicator for screening of diabetes in the general population.

Conclusion

It has been observed that NLR ratio value are higher in subjects with new onset type-2 diabetics. It has been also observed that NLR values are relatively higher in Indian population than western countries. A positive correlation between Neutrophils lymphocyte ratio and type-2 DM, so NLR ratio should be used as prognostic and disease monitoring tool during the follow up diabetic patients like HbA1c, further studies are still needed to confirm present results.

Conflict of Interest: Nil

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